EFFICACY OF INIMEX PEPTIDE SEQ ID No.7 IN ANIMAL MODELS OF BACTERIAL INFECTION

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ABSTRACT & CONCLUSIONS

The efficacy of SEQ ID No.7 has been demonstrated in mouse models representative of abdominal infections, surgical wounds, pneumonia, cancer chemotherapy and life threatening inflammation. This work included infections with normal and drug resistant bacteria, Gram-negative and Gram-positive organisms, and prophylactic or therapeutic administration. SEQ ID No.7 rapidly activates the innate response and is quickly degraded in biological fluids. The activation persists for at least 48 hours in mice.

The impact of SEQ ID No.7 on innate defences is evident in animals that lack T and B cells and also in neutropenic animals. The prototype drug is safe when used in combination with frontline antibiotic therapy and the combination of these two independent treatment approaches leads to an increased efficacy of treatment.

SEQ ID No. 7 substantially reduces the inflammatory cytokine response to infection, suggesting that Innate Defence Regulator drugs will offer symptomatic benefit as well as reduced probability of infections progressing to systemic inflammation – ("sepsis").

This document summarizes the data supporting these conclusions.

INTRA-PERITONEAL INFECTION MODELS

The intra-peritoneal (IP) infections summarized here are *in vivo* models in which the bacteria of interest are injected into the peritoneal cavity of mice[1]. Depending upon the specific pathogen, the infection may pass from the fluid within the peritoneal cavity to the blood and become a systemic infection (bacteria may be observed in the blood within 2-4 hours). Performance of a test article in an IP infection model can be evaluated by assessing bacterial counts (colony forming units – CFU) following administration or by considering relative survival. Clinical signs such as body temperature are used as secondary endpoints in these models.

This model is routinely used in antibiotic drug development as a rapid screening model for bacterial killing ability. While innate defence peptides do not function by direct bacterial killing, efficacy in this model is still an important demonstration of the ability of the peptide to induce a "protective" host response in a very acute and rapid infection.

Test compound can be administered in an IP infection model by injection into the peritoneal cavity (IP), into the tail vein (directly into blood, intravenous – IV), into muscle (intra-muscular – IM) or beneath the skin (sub-cutaneously - SC). Inimex has primarily investigated the IP route, with some studies examining IV administration. Treatment can also be administered prophylactically (prior to infection), concomitantly with infection, or therapeutically (after infection). Due to the rapidly developing acute nature of the infection in this model, therapeutic

[1] Weiss WJ, Murphy T, Lenoy E, Young M. In vivo efficacy and pharmacokinetics of AC98-6446, a novel cyclic glycopeptide, in experimental infection models. Antimicrob Agents Chemo. 2004; 28(5); 1708-1712.

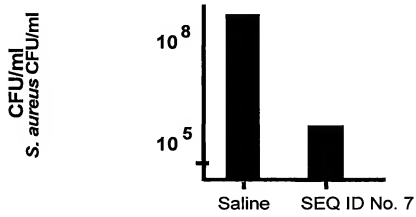
efficacy with either antibiotics or Inimex compounds is tested within 4 to 6 hours of initiation of infection.

S. aureus model

S. aureus is one of the most common causes of nosocomial and community-acquired infections and has developed stable resistance to many antibiotics including methicillin and vancomycin (reviewed in Smith et al. 1999)[2].

A typical experiment in the therapeutic model is shown in Figure 1. Female ICR mice were injected IP with 24 mg/kg SEQ ID No.7 four hours after *S. aureus* 25923 (9.3×10^9 cfu) had been injected in a 5% mucin suspension. The animals were sacrificed 24 hours after infection and the numbers of viable bacteria were assessed in peritoneal lavage fluid. Even though SEQ ID No.7 has no direct bacterial activity, the numbers of recovered viable bacteria were reduced by approximately 3 orders of magnitude.

Figure 1. SEQ ID No.7 in IP *S. aureus* model – bacterial counts



It is notable that the animals could be rescued from infection by a peptide injection 4 hours after infection – at a time when only very aggressive antibiotic treatment would be effective, due to the rapid proliferation of the pathogen. In other experiments (data not shown), the peptide has been effective when administered as late as 6 hours after infection. The mobilization of innate immune defences by the peptide must therefore occur rapidly.

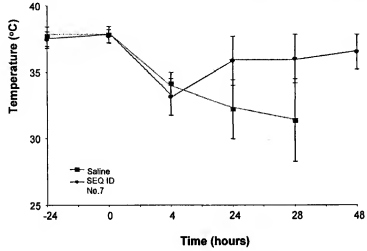
S. aureus is a gram positive organism. Similar data have been obtained in animals infected intra-peritoneally with the gram negative organism, entero-hemorrhagic *E. coli* (EHEC). Data of this type reveal the broad spectrum activity of SEQ ID No.7, as expected for an agent that accentuates the defence system of the host organism rather than directly impacting the pathogen.

Mice infected with S. aureus display reduced body temperature over time – leading to death. Figure 2 shows that the body temperature of animals treated with SEQ ID No.7 four hours

[2] Smith TL, Pearson ML, Wilcox KR, Cruz C, Lancaster MV, Robinson-Dunn B, Tenover FC, Zervos MJ, Band JD, White E, Jarvis WR. Emergence of vancomycin resistance in *Staphylococcus aureus*. Glycopeptide-Intermediate *Staphylococcus aureus* Working Group. N Engl J Med. 1999 18;340(7):493-501.

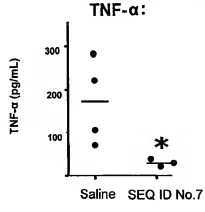
after infection recovered substantially by 24 hours, consistent with the reduction in numbers of bacteria and also with the reduction in levels of the inflammation-associated cytokine, TNF-α, in peritoneal fluids (Figure 3).

Figure 2. SEQ ID No.7 in IP *S. aureus* model – body temperature



Mice were implanted with microchips 4d before the study was initiated. Mice were then injected IP with *S. aureus* in 5% mucin, **4 h later** SEQ ID No.7 (24 mg/kg) was injected. The temperature of the mice was recorded over the length of the study. All animals in the saline treated group died by 48 h.

Figure 3. SEQ ID No.7 in IP *S. aureus* model – peritoneal TNF-α



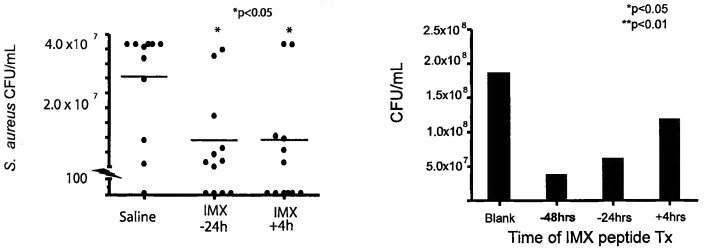
Mice injected IP with S. aureus in 5% mucin. 4 h later, peptide injected. Mice sacrificed 18 h later and the bacterial load, TNF-α and cell counts in peritoneal lavage fluid were assessed. Data from individual mice are shown.

Prophylaxis and Therapy; Pharmacodynamic implications

A prophylactic approach was explored in numerous experiments. A single injection of SEQ ID No.7 was administered either 48 hours or 24 hours prior to infection. Figure 4 shows the results of two such investigations, illustrating the effectiveness of the peptide in this regime. Although the

pharmacokinetic half life of the drug is short, its biological effect (pharmacodynamics) have an extended lifetime.

Figure 4. SEQ ID No.7 as a prophylactic in the IP *S. aureus* model

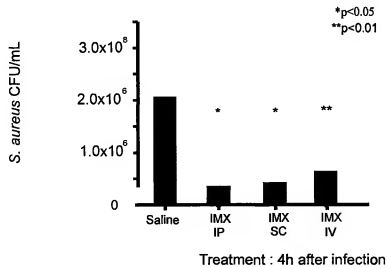


SEQ ID No. 7 at 24 mg/kg was administered to female ICR mice at the indicated times before or after infection with S. aureus 25923 in 5% mucin. N=12 per group. Animals were sacrificed 24 hours after infection and bacterial counts were measured in peritoneal washings. Two separate experiments are shown. In the left panel, counts from individual mice are shown. Means are shown in the right panel.

Routes of Administration

A number of routes of administration of injectable SEQ ID No. 7 have been tested. Figure 5 shows data from a comparison of intraperitoneal, subcutaneous and intravenous administration of the peptide at 24 mg/kg, 4 hours after intraperitoneal infection with S. aureus in 5% mucin. All routes were effective, with differences between the routes being insignificant.

Figure 5. Comparison between routes of injection of SEQ ID No. 7

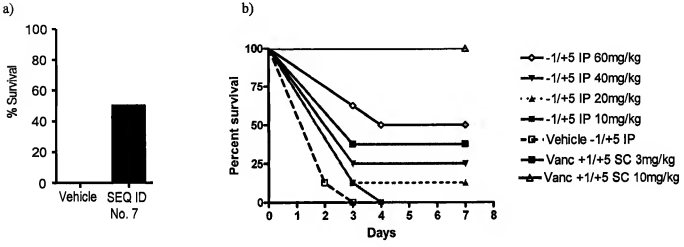


Female ICR mice; n=8 per group

Antibiotic Resistance

Since SEQ ID No.7 has no direct anti-microbial activity, we hypothesized that its actions would be independent of antibiotic resistance in the bacteria. It is known that intraperitoneal infection of mice with MRSA (a methicillin-resistant strain of S. aureus (ATCC 33591) can be controlled with high doses of subcutaneous vancomycin. In our hands, 38% survival was achieved with 3mg/kg vancomycin administered at 1 and 5 hours after infection, while 100% survival was achieved with 10 mg/kg doses of vancomycin. These vancomycin doses are high compared with those used in human clinical practice, emphasizing the aggressive nature of the MRSA model. In one study (Figure 6a), a 60 mg/kg dose of SEQ ID No.7 given 24 hours fection protected 50% of the mice. In another study (Figure 6b) the dose-responsiveness of this protection was demonstrated – in this experiment SEQ ID No.7 was administered twice, at 1 hour before and 5 hours after infection.

Figure 6. SEQ ID No. 7 is active against MRSA



Independent of Adaptive Immune System

The hypothesis was tested that the stimulation of innate defences achieved by SEQ ID No. 7 would be independent of neutrophils and of T and B lymphocytes. RAG-1 transgenic mice, severely deficient in cells of the T and B lineages, could be protected from *S. aureus* infection by SEQ ID No. 7 injection (Figure 7a). In other experiments, neutropenia was induced in mice by two treatments with cyclophosphamide. SEQ ID No. 7, administered 4 hours after infection of the mice with *S. aureus*, doubled their survival at 24 hours (Figure 7b).

Figure 7. SEQ ID No. 7 is active in immune-deficient mice

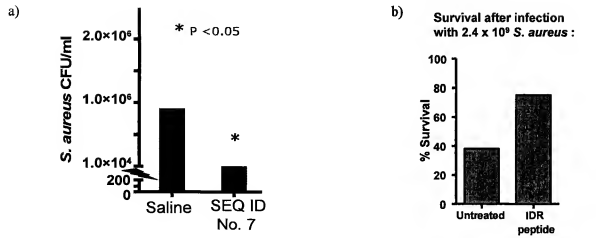


Figure 7a: Female RAG1 mice (lacking T and B cells) were injected IP with S. aureus in 5% mucin. 4 h later, 24 mg/kg SEQ ID No. 7 was injected. Mice were sacrificed 24h later and the bacterial load in peritoneal lavage fluid was assessed.
Figure 7b: Female CD1 mice were rendered neutropenic by treatment with 200mg/kg cyclophosphamide 4 and 1 day before intraperitoneal infection with S. aureus 25923 in 5% mucin. 24 mg/kg SEQ ID No. 7 was administered IP 4 hours after infection and survival was assessed at 24 hours. N=8

These studies clearly show that SEQ ID No. 7 modulation of innate defence does not rely on the presence of effector cells of the adaptive immune system.

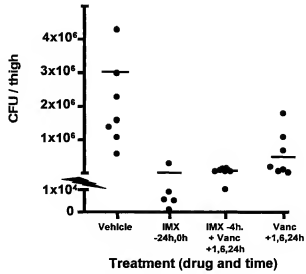
THIGH ABCESS MODEL

The thigh abscess infection model results in a walled-off infection as a consequence of embedding beads coated with bacteria into the thigh[3]. The *S. aureus* for this model was originally isolated from a clinical wound infection and is often used in rodent thigh models used to test antibiotics[4]. This model is commonly used in antibiotic screening as a more rigorous infection which can persist over longer timeframes and which can be used to probe more detailed pharmacokinetic attributes of the test article. Nevertheless, dose requirements for this model are not considered predictive of dose requirements for human treatments.

The thigh abscess model is very difficult to treat with antibiotics and the positive control, vancomycin, is usually administered at 100 mg/kg three times (1, 6 and 24 hours after infection). This dose is significantly higher than the 10 mg/kg used in the IP infection models and well in excess of the human dose of 10 mg per adult. In comparison, in these studies IMX503 has been administered once or twice (as specified), (Figure 8) and yields a comparable impact on the infection.

[3] Ford CW, Hamel JC, Stapert D, Yancey RJ. Establishment of an experimental model of a *Staphylococcus aureus* abscess in mice by use of dextran and gelatin microcarriers. J Med Microbiol. 1989 Apr;28(4):259-66.
[4] Louie A, Kaw P, Liu W, Jumbe N, Miller MH, Drusano GL. Pharmacodynamics of daptomycin in a murine thigh model of *Staphylococcus aureus* infection. Antimicrob Agents Chemother. 2001 45(3):845-51.
Fetiye K, Karadenizli A, Okay E, Oz S, Budak F, Gundes S, Vahaboglu H. Comparison in a rat thigh abscess model of imipenem, meropenem and cefoperazone-sulbactam against *Acinetobacter baumannii* strains in terms of bactericidal efficacy and resistance selection. Ann Clin Microbiol Antimicrob. 2004 8;3:2.

Figure 8. SEQ ID No.7 is active in *S. aureus* thigh abcess



Female Swiss albino mice; n=8; IMX=SEQ ID No.7 (100mg/kg)

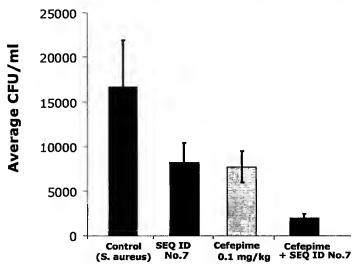
Complementarity with Antibiotics

Since SEQ ID No.7 has no direct anti-microbial activity, we hypothesized that the actions of an antibiotic and those of SEQ ID No.7 would be complementary since they would act on the bacteria through different routes. We tested SEQ ID No.7 + antibiotic combination therapy in two infection models: intraperitoneal and thigh abcess infection.

Figure 8 illustrates the results of an experiment in the thigh abcess model, where SEQ ID No.7 was administered at the high dose of 100 mg/kg in the infected thigh at the times indicated. Another group of mice was treated with high dose vancomycin and one group received combination treatment. Since both the vancomycin alone and the SEQ ID No.7 alone were effective at these doses, no additive effects could be detected in the combination therapy group.

In an alternative experimental design, sub-therapeutic doses of SEQ ID No.7 and/or Cefepime were administered to mice 6 hours after intraperitoneal infection with *S. aureus*. Figure 9 shows that the combination treatment was more effective than either sub-optimal treatment alone. These data suggest that SEQ ID No.7 might offer benefit when antibiotic resistance begins to emerge and antibiotic treatment becomes suboptimal.

Figure 9. SEQ ID No.7 complements antibiotic treatment



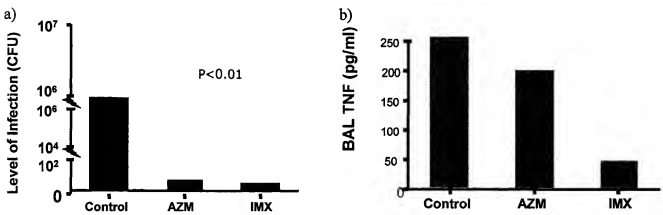
IP infection model in female IDR mice. N=8.

PNEUMONIA MODEL

Bacterial pneumonia is a leading cause of morbidity and mortality in both developed and developing countries. Morbidity may result from destruction of lung tissue during infection, with subsequent scarring. Affected areas may be incapable of gas exchange, reducing respiratory reserve.

On the basis of published reports of animal models[5,6], a murine pneumonia model utilizing intranasal delivery of *Streptococcus pneumoniae* (ATCC 6303) was selected for evaluation of Inimex peptides. SEQ ID No.7 was administered intra-peritoneally to mice at 24 mg/kg, at the time of infection or 24 hours later and mice were sacrificed 48 hours after infection. The broncheoalveolar lavage (BAL) fluid was collected and plated for viable bacterial counts. A strong inhibition of broncho-pulmonary CFU numbers was observed (Figure 10a). The levels of the inflammatory cytokine, TNF-α, were also measured in BAL fluid. Notably, TNF-α levels were markedly reduced in peptide-treated animals whereas azithromycin-treated animals had TNF-α levels similar to those of untreated controls.

Figure 10. SEQ ID No.7 is effective against *S. pneumoniae*- induced inflammation



At time 0 all mouse groups were infected intra-nasally with 1.2×10^6 of bacterial CFUs. 24 mg/kg of SEQ ID No.7 was administered intra-peritoneally at the same time. In another group, AZM was administered sub-cutaneously at 24 mg/kg 24 hours later. 48 hours after infection all mouse groups were sacrificed and BAL fluid was collected.
a) Bacterial counts in BAL fluid. b) TNF-α levels in BAL fluid. The geometric mean for each group is shown.

[5] Dallaire F, Ouellet N, Simard M, Bergeron Y, Bergeron MG. (2001) Efficacy of recombinant human granulocyte colony-stimulating factor in a murine model of pneumococcal pneumonia: effects of lung inflammation and timing of treatment. J Infect Dis. 183:70-7.

[6] Bast DJ, Yue M, Chen X, Bell D, Dresser L, Saskin R, Mandell LA, Low DE, de Azavedo JC. (2004) Novel murine model of pneumococcal pneumonia: use of temperature as a measure of disease severity to compare the efficacies of moxifloxacin and levofloxacin. AAC 48:3343-8.